Asian Journal of Applied Science and Technology (AJAST)
Volume 9, Issue 2, Pages 54-65, April-June 2025

Impact of Selected Food Preservatives and Fruit Ripening Agents on Oxidation of Normal Human Hemoglobin

Emmanuel Ikechukwu Nnamonu[1], Edmund Chidiebere Mbegbu[2] & Chiemekam Samuel Ezechukwu[3*]

[1]*Department of Biology, Federal College of Education, Eha-Amufu, Enugu State, Nigeria.* [2]*Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Enugu State, Nigeria.* [3]*Drug Discovery and Biotechnology Unit, Lion Science Park, University of Nigeria, Nsukka, Nigeria. Corresponding Author (Chiemekam Samuel Ezechukwu) Email: chiemekam.ezechukwu.pg76663@unn.edu.ng**

DOI: https://doi.org/10.38177/ajast.2025.9206

Article Received: 07 March 2025 *Article Accepted: 17 May 2025* *Article Published: 26 May 2025*

ABSTRACT

The use of unauthorized quantities of food processing substances/preservatives and fruit ripening agents has raised significant apprehension regarding potential health repercussions. This study evaluated the effect of selected food preservatives and fruit ripening substances, including calcium carbide, bifenthrin, ethanol, ethylene glycol, potassium carbonate, and monosodium glutamate, on oxy-hemoglobin concentration and oxidation of hemoglobin. Each food preservative was tested at two concentrations: 5 mg/ml and 2.5 mg/ml. The effects of the preservatives on oxy-hemoglobin concentration and oxidation of normal hemoglobin were determined using UV-spectrophotometry following the standard protocols. Exposure to the different food preservatives led to a significant ($p<0.05$) dose-dependent decrease in oxy-hemoglobin concentrations. Notably, a high dose of bifenthrin led to zero oxy-hemoglobin concentration. Concentration and time-dependent reductions were observed in the absorbance maxima of oxy-hemoglobin reacted with the high (5 mg/ml) and low (2.5 mg/ml) doses of the different food preservatives, with bifenthrin showing the greatest effect. The study demonstrated significant variations in oxy-hemoglobin concentration in response to diverse food preservatives/fruit-ripening substances, contributing to ongoing studies on the potential effects of these substances on human health.

Keywords: Food Preservatives; Fruit Ripening Agents; Hemoglobin Oxidation; Human Hemoglobin; Bifenthrin; Calcium Carbide; Ethylene Glycol; Food Additives; Monosodium Glutamate; Oxy-Hemoglobin.

1. Introduction

In the food industry, food preservatives, processing substances, and fruit ripening agents are widely utilized to enhance the longevity, quality, and accessibility of diverse food items [1]. Preservation and ripening help to ensure the availability of fresh and visually appealing fruits year-round through the use of various preservatives and food processing substances that enhance the ripening process, extend shelf life, and maintain fruit quality. Nevertheless, the excessive and uncontrolled usage of these substances has raised significant apprehension regarding potential health repercussions [2].

Research has documented the negative impacts of food preservatives, processing agents, and fruit-ripening substances. The utilization of carbide, for instance, poses serious health risks when inhaled. Exposure to calcium carbide as a fruit ripening agent, can lead to skin irritation, resulting in rashes and redness. Additionally, it can cause lung irritation, leading to coughing and shortness of breath [3]. Calcium carbide's reaction with moisture releases phosphine gas (PH_3), which is known to be toxic to the respiratory system [4].

Bifenthrin is a pyrethroid insecticide, often used in preserving food products. Recent studies have raised concerns about the potential immunotoxin and proinflammatory effects of bifenthrin and other pyrethroids in vertebrates, attributable to oxidative stress. Bifenthrin metabolism involves the participation of glutathione-S transferases (GSTs). Non-target organisms experience oxidative stress when bifenthrin and other pyrethroids competitively inhibit GSTs in their livers [5],[6]. The consumption of ethanol remains an undisputed risk factor for preventable diseases worldwide. According to a report by the World Health Organization (WHO), harmful alcohol (ethanol) use

accounted for 5.3% of all global deaths in 2016 [7]. Ethylene has been recognized as preceding increased levels of stress-associated hormones and inflammatory cytokines in the bloodstream of humans [8].

An examination of relevant published articles reveals a significant research gap regarding the hematological effects of the selected preservatives and food processing substances on human subjects. The majority of available studies have predominantly investigated the acute toxicity and potential long-term health effects of these substances, while the specific impact on hematological parameters in healthy individuals remains largely unexplored. Furthermore, although some studies have examined the individual impact of preservatives or food processing substances on human health, there is a lack of comprehensive research investigating the combined effects of multiple substances. The simultaneous presence of these substances in various fruit products raises concerns about potential additive or synergistic effects that may affect hematological parameters [9].

Therefore, it is crucial to investigate the potential effects of these substances on human health, particularly their impact on hematological parameters in healthy individuals. This study evaluated the influence of selected preservatives and food processing substances, namely calcium carbide, bifenthrin, ethanol, ethylene, potassium carbonate and sodium glutamate on oxy-hemoglobin levels and oxidation of hemoglobin.

1.1. Study Objectives

1. To evaluate the influence of calcium carbide on oxy-hemoglobin concentration and the rate of hemoglobin oxidation in normal human blood samples.

2. To determine the effect of bifenthrin exposure on oxy-hemoglobin levels and the extent of hemoglobin oxidation in healthy human erythrocytes.

3. To assess how ethanol treatment alters oxy-hemoglobin content and promotes oxidative conversion of hemoglobin in vitro.

4. To investigate the impact of ethylene on oxy-hemoglobin stability and the degree of hemoglobin oxidation in normal human blood.

5. To measure the changes in oxy-hemoglobin concentration and hemoglobin oxidation induced by potassium carbonate in healthy human erythrocytes.

6. To quantify the effect of sodium glutamate on oxy-hemoglobin levels and its potential to catalyze hemoglobin oxidation in normal human blood samples.

2. Materials and Methods

2.1. Reagents

Analytical grades of reagents were used. The reagents used include: sodium metabisulphite (2%), concentrated hydrochloric acid (35%), methylated spirit (95%), potassium phosphate (97%), and sodium chloride (39.4%) procured from Panhong Chemical Co., Shenzhen, Guangdong, China, Sephadex G-150 from Thomas Scientific, USA and Giemsa stain from Sigma-Aldrich, Inc., USA.

2.2. Equipment and Instrument

Water bath, digital weighing balance (Metler H_3O, Switzerland), Whatman No 1 filter paper, pH meter, Moticam digital camera 2.0 and Olympus BH-2 microscope (USA), ultraviolet-visible spectrophotometer (V-750), rotary evaporator, autoclave, refrigerator, glass wool and Incubator (Beckman Coulter Co., USA).

2.3. Blood Collection

Ethical approval and informed consent were obtained from the Faculty of Biological Sciences Research Ethics Committee, University of Nigeria Nsukka, and the blood donor, respectively. Whole blood samples (5 ml) were collected through vein puncture using Venoject tubes containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant.

2.4. Blood Sample Preparation for Spectral Study

The collected whole blood samples were centrifuged at 3000 g for 30 minutes at a temperature of 4˚C to separate the red blood cells. Subsequently, the supernatant (serum) was discarded, and the red blood cells were washed thrice with an ice-cold phosphate buffer (0.1M, pH 7.4). Then, ice-cold distilled water (4 ml) was added to lyse the red blood cells, followed by centrifugation for 30 minutes at 5000 g and 4˚C [10].

2.5. Experimental Design for Spectral Study

The experiment consists of the following groups:

Group 1: Hemoglobin A (HbA) without test material (Normal control)

Group 2: HbA treated with 200 µl of high-dose (5 mg/ml) Calcium carbide

Group 3: HbA treated with 200 µl of low-dose (2.5 mg/ml) Calcium carbide

Group 4: HbA treated with 200 µl of high-dose (5 mg/ml) Bifenthrin

Group 5: HbA treated with 200 µl of low-dose (2.5 mg/ml) Bifenthrin

Group 6: HbA treated with 200 µl of high-dose (5 mg/ml) Ethanol

Group 7: HbA treated with 200 µl of low-dose (2.5 mg/ml) Ethanol

Group 8: HbA treated with 200 µl of high-dose (5 mg/ml) Ethylene glycol

Group 9: HbA treated with 200 µl of low-dose (2.5 mg/ml) Ethylene glycol

Group 10: HbA treated with 200 µl of high-dose (5 mg/ml) Potassium carbonate

Group 11: HbA treated with 200 µl of low-dose (2.5 mg/ml) Potassium carbonate

Group 12: HbA treated with 200 µl of high-dose (5 mg/ml) Monosodium glutamate

Group 13: HbA treated with 200 µl of low-dose (2.5 mg/ml) Monosodium glutamate

2.6. Hemoglobin Purification

Sephadex G-200 was swollen in phosphate buffer for three days and then washed with distilled water until reaching a neutral pH. The gel was packed into a glass column, and the column was equilibrated with phosphate buffer.

Hemolysate was loaded onto the column and eluted at 4°C using a linear gradient of the buffer. The elution was collected until the absorbance at 280 nm reached zero. The Oxy-hemoglobin (Oxy-Hb) was collected and stored at −18°C for subsequent use within 24 hours [10].

2.7. UV Absorption Spectral Study

The UV-visible absorption spectra of the treatment groups and control were recorded using a V-750 UV-visible spectrophotometer. The spectra were determined within the range of 400–700 nm using a quartz cuvette with a 1.0 cm path length.

2.8. Measurement of Oxy-Hb Concentration

The absorbance spectra were scanned within the range of 400 to 700 nm. Oxy-Hb concentrations were expressed in millimolar (mM) units, and the extinction coefficients for oxy-Hb species were derived from previous studies by Meng and Alayash [11]. The concentrations were obtained by calculation from the equation: C = A/el, with C being the concentration; A, the absorbance at specific wavelengths; e, the extinction coefficient, and l, the cuvette length.

3. Results and Discussion

3.1. Effect of different food additives and preservatives on oxy-Hb concentration

The result revealed that the exposure of red blood cells to the different food additives and preservatives produced notable decreases in oxy-Hb concentrations. The tested concentrations of different food additives and preservatives elicited significant ($p<0.05$), concentration-dependent decreases in oxy-Hb concentrations, especially with bifenthrin at 5 mg/ml which completely depleted oxy-Hb concentrations (Table 1).

Table 1. Effect of different food additives and preservatives on oxy-Hb concentration

Group	Oxy-Hb concentration (mmol/L)	
	Low dose (2.5 mg/ml)	High dose (5 mg/ml)
Normal oxy-Hb	0.0690	0.0690
Calcium carbide	0.0025	0.0010
Bifenthrin	0.0050	0.0000
Ethanol	0.0050	0.0010
Ethylene glycol	0.0075	0.0005
Potassium carbonate	0.0045	0.0010
Monosodium glutamate	0.0040	0.0010

There was a concentration and time-dependent reduction in absorbance maxima of oxy-Hb reacted with high and low doses of the different food additives and preservatives as compared with the Control. The observed depletion of oxy-Hb could be due to decreased oxygen affinity in the hemoglobin molecules. Worthy of note is that high doses of the different food additives and preservatives especially bifenthrin had the highest reduction in the absorbance maxima of the reacted oxy-Hb. These findings are in consonance with some other studies investigating the effects of food additives and preservatives on oxy-Hb concentration and absorbance maxima [12],[13]. Concerns have been raised regarding the potential effects of food additives and preservatives on human health especially their

impact on oxyhemoglobin (oxy-Hb) concentration and absorbance maxima, which are important indicators of oxygen-carrying capacity and oxygen saturation in the blood [12],[13].

3.2. Effects of high and low doses of different food additives and preservatives on oxidation of human hemoglobin

The typical UV-visible spectrum of unreacted oxy-Hb showed two distinct absorbance peaks at 541 nm (beta band) and 576 nm (alpha band) (Figure 1). Against this baseline observation, the effects of high and low doses of different food additives and preservatives on the oxidation of hemoglobin A (HbA) were determined. The findings revealed concentration-dependent decreases in the absorbance peaks of oxy-Hb at both concentrations (2.5 mg/ml and 5mg/ml) of the different food additives and preservatives tested (Figures 2–7). The 5 mg/ml concentration of the different food additives and preservatives especially Bifenthrin, showed the highest reduction in the absorbance peaks of the reacted oxy-Hb (Figure 3a and b).

Preservatives and food processing substances are intentionally used products in the food industry. When used in authorized quantities, “these substances increase the durability of the food product and enhance or modify its properties, including its appearance and flavor or structure, without diminishing the nutritional value but the use of some might pose risks to human health” [14].

Our findings on the effects of high and low concentrations of different food additives and preservatives on the oxidation of human hemoglobin suggest a potential negative impact on oxygen transport in the blood. In a similar study, it was discovered a shift in the absorbance maxima, suggested alterations in the oxygen-binding properties of oxy-Hb due to the presence of preservatives [15]. Furthermore, the current literature was summarized on the effects of food additives and preservatives on oxy-Hb concentration and absorbance maxima. The review highlighted the diverse range of additives and preservatives that have been found to impact oxy-Hb parameters, including artificial colors, flavor enhancers, and antioxidant additives [16].

The present study's findings demonstrate significant variations in oxy-Hb concentration in response to diverse food additives and preservatives, warranting further investigation into their potential impacts on human health.

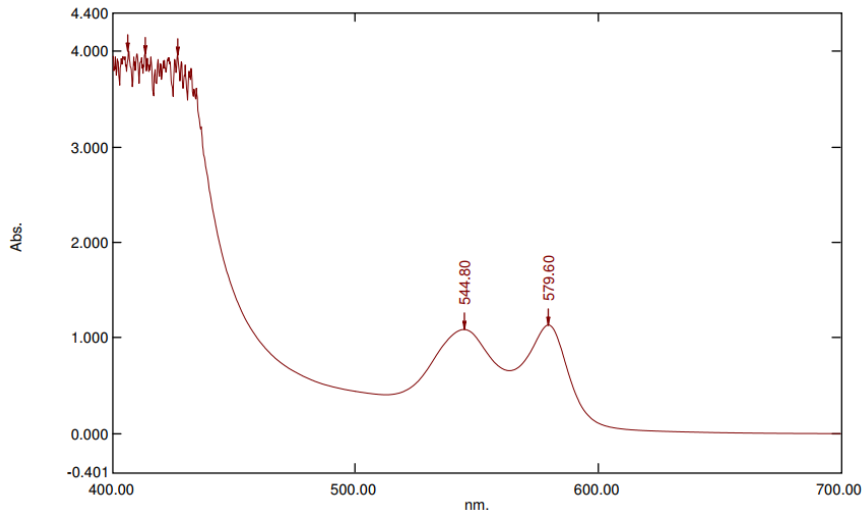

Figure 1. Spectra characterization of unreacted oxy-Hb from Control Group

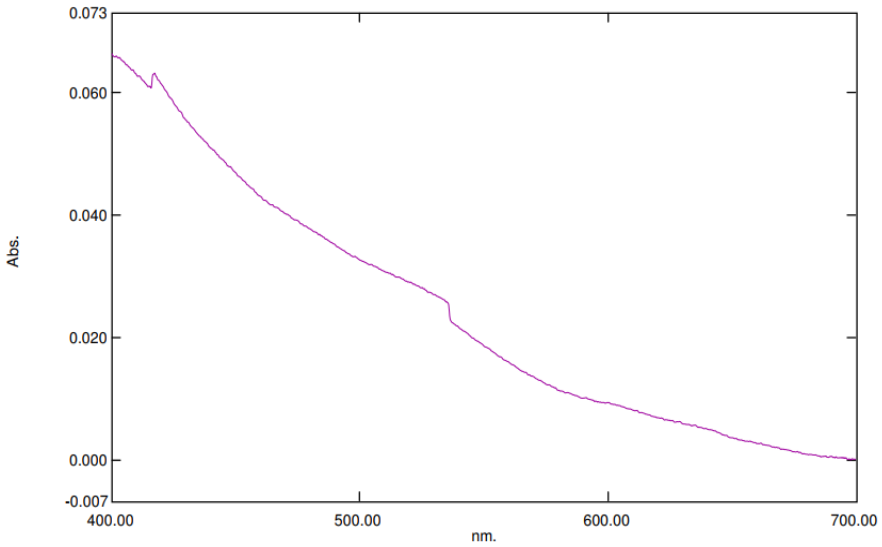

Figure 2a. The effect of a high concentration of calcium carbide on the oxidation of hemoglobin

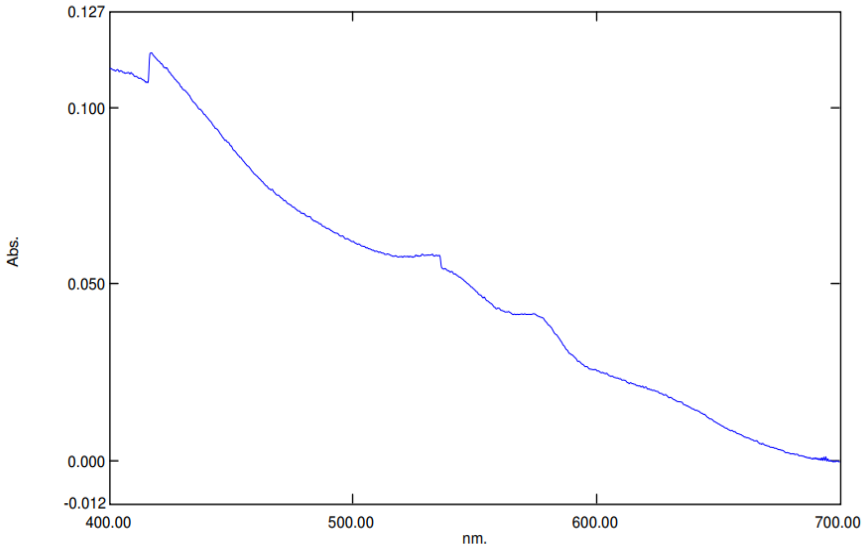

Figure 2b. The effect of a low concentration of calcium carbide on the oxidation of hemoglobin

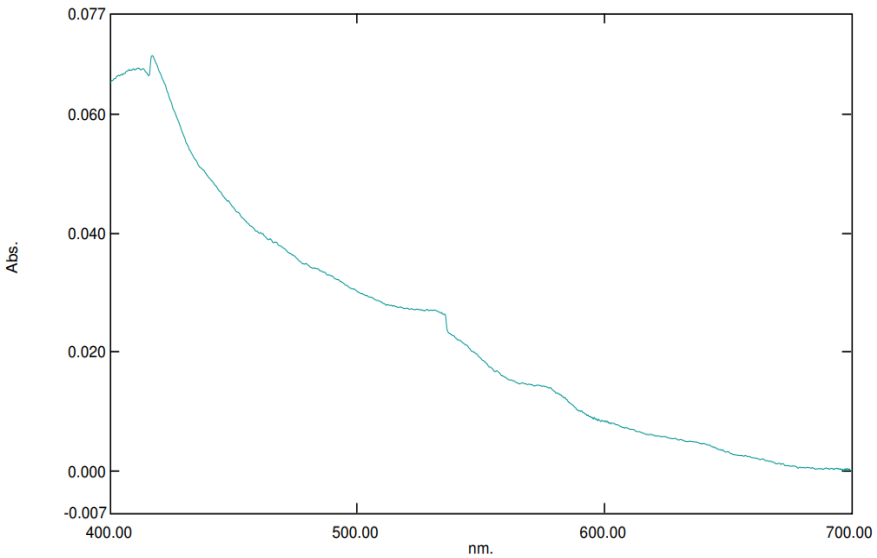

Figure 3a. The effect of a high concentration of Bifenthrin on the oxidation of hemoglobin

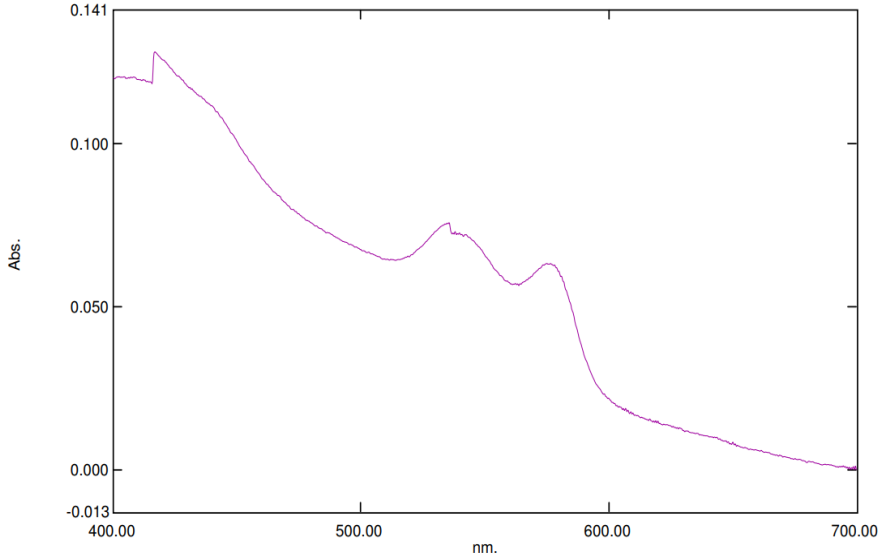

Figure 3b. The effect of a low concentration of Bifenthrin on the oxidation of hemoglobin

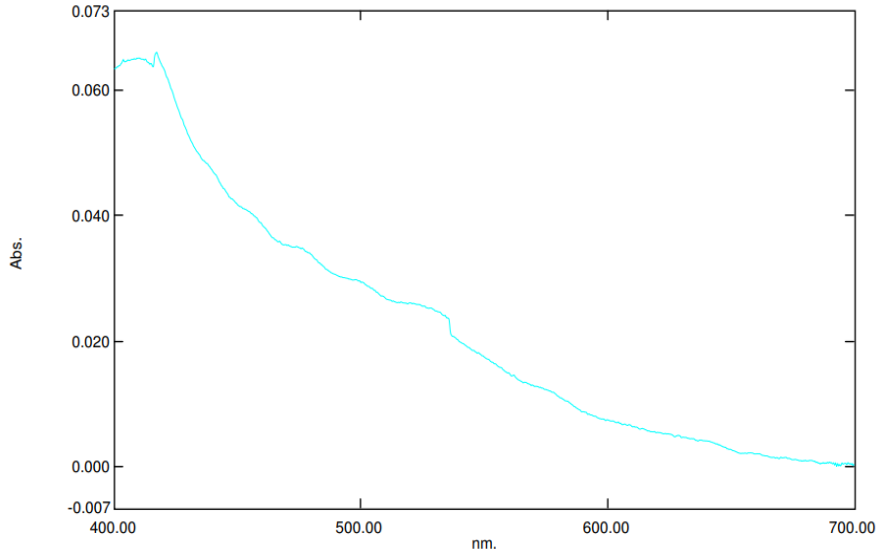

Figure 4a. The effect of a high concentration of ethanol on the oxidation of hemoglobin

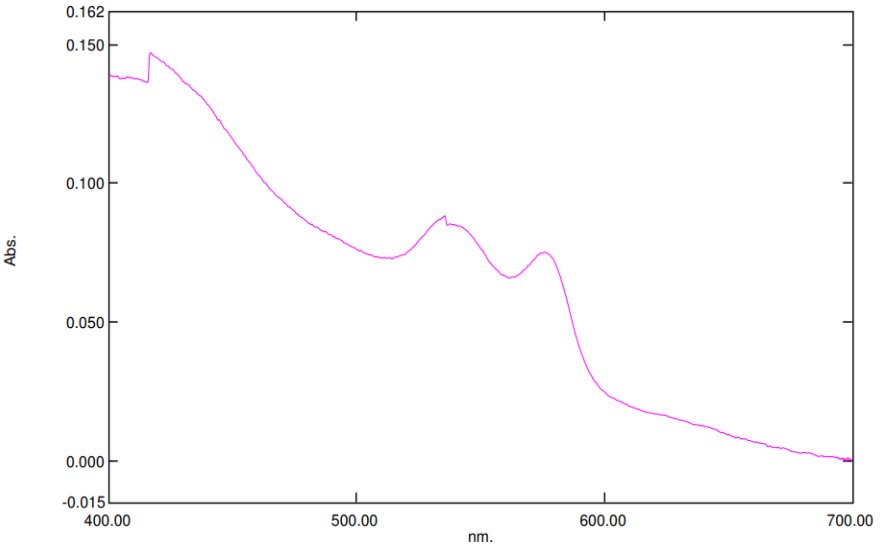

Figure 4b. The effect of a low concentration of ethanol on the oxidation of hemoglobin

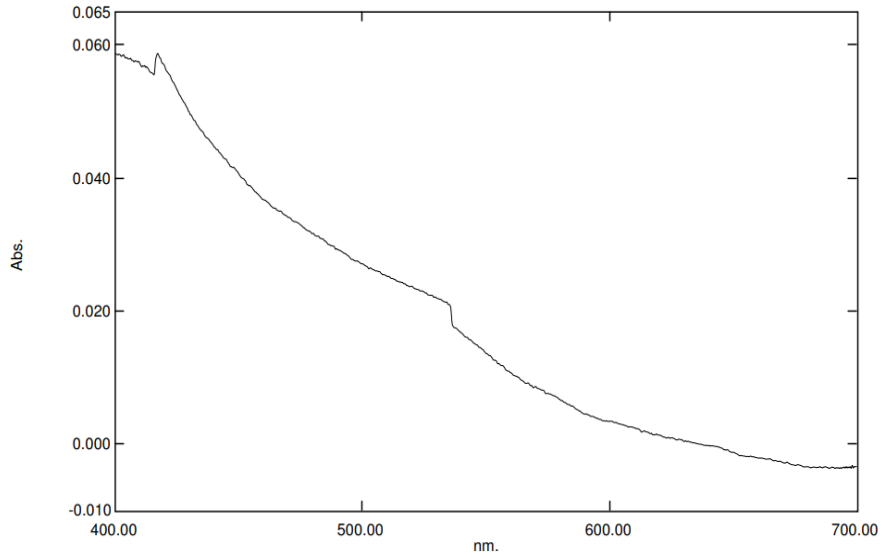

Figure 5a. The effect of a high concentration of ethylene glycol on the oxidation of hemoglobin

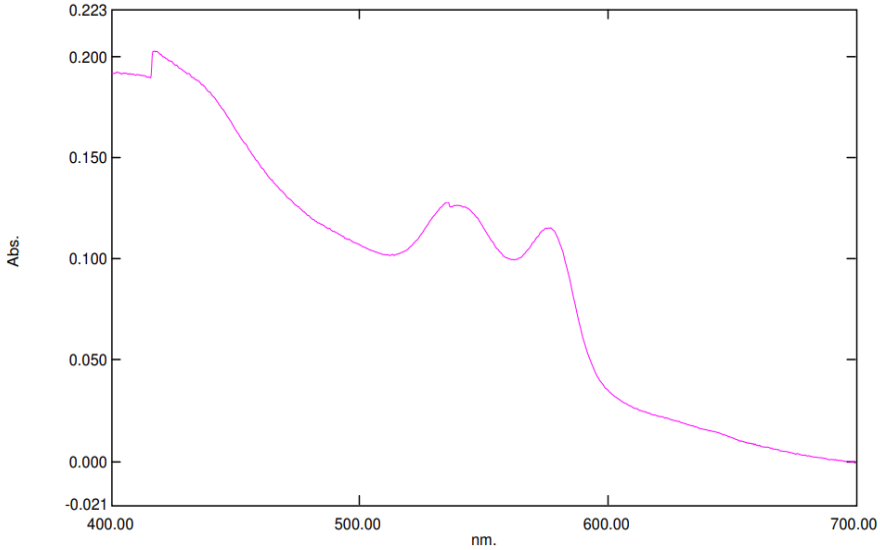

Figure 5b. The effect of a low concentration of ethylene glycol on the oxidation of hemoglobin

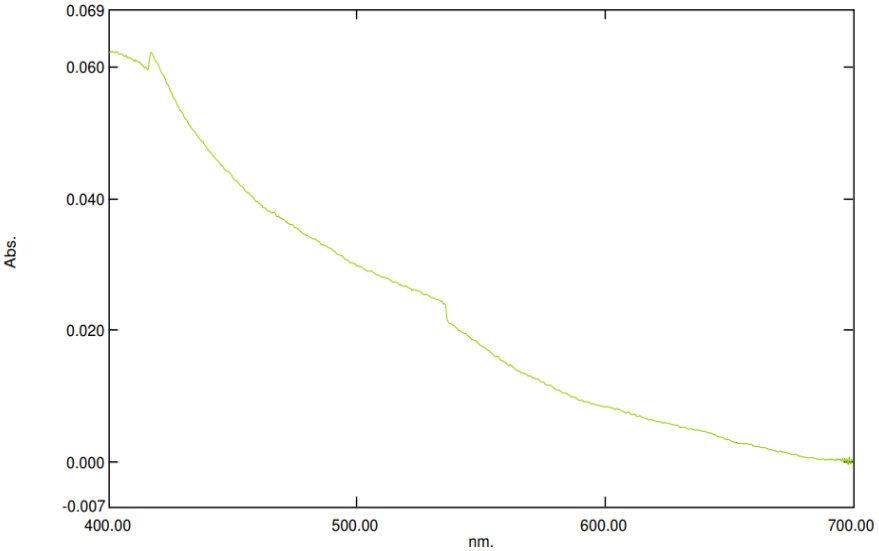

Figure 6a. The effect of a high concentration of potassium carbonate on the oxidation of hemoglobin

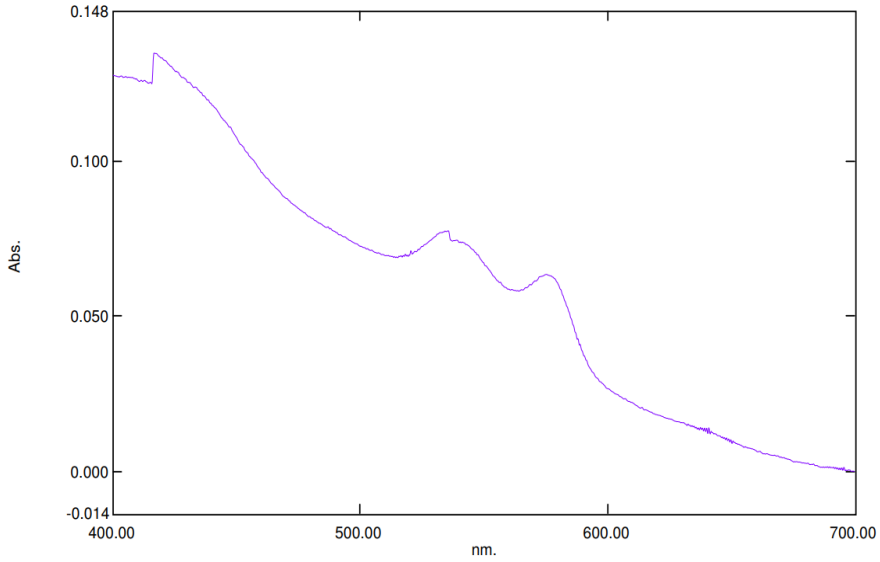

Figure 6b. The effect of a low concentration of potassium carbonate on the oxidation of hemoglobin

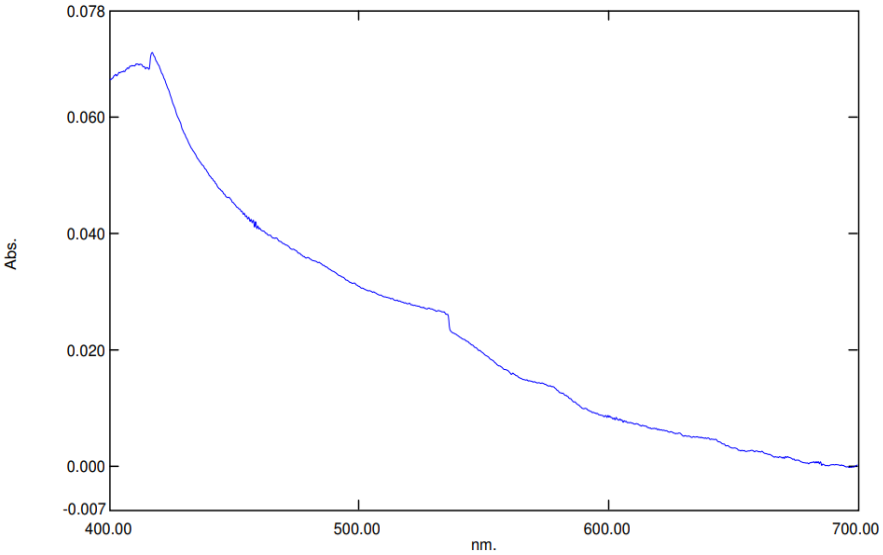

Figure 7a. The effect of a high concentration of monosodium glutamate carbonate on the oxidation of hemoglobin

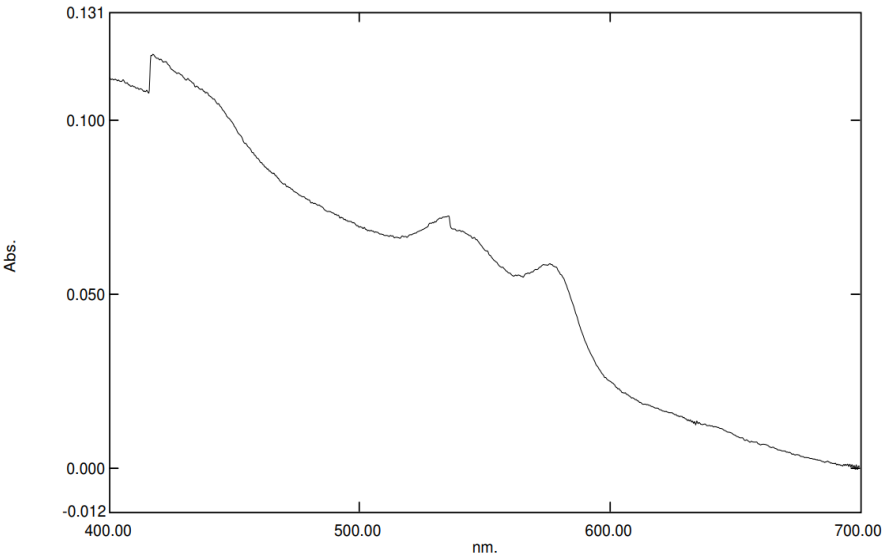

Figure 7b. The effect of a low concentration of monosodium glutamate carbonate on the oxidation of hemoglobin

4. Conclusion

The different food additives and preservatives at the applied doses (5 mg/ml and 2.5 mg/ml) caused a significant concentration-dependent decrease in oxy-Hb concentrations, with Bifenthrin at 5 mg/ml resulting in complete oxy-Hb depletion. Ethylene glycol, calcium carbide, potassium carbide, sodium glutamate and ethanol also depleted oxy-Hb but not to a zero level. The impact of food additives and preservatives on hemoglobin oxidation revealed their potentials to significantly deplete oxy-hemoglobin in red blood cells. Notably, Bifenthrin showed the greatest impact in this regard. These food additives and preservatives, therefore, have a potential adverse impact on human health.

5. Future Suggestions

1. Future studies should include in vivo experiments to evaluate the systemic hematological effects of chronic, low-level exposure to these preservatives and ripening agents in animal models.

2. Researchers should examine potential additive or synergistic interactions by testing mixtures of two or more of these substances at concentrations relevant to real-world exposures.

3. It will be important to investigate the molecular mechanisms underlying hemoglobin oxidation—such as reactive oxygen species generation and antioxidant enzyme responses—using advanced spectroscopic and biochemical assays.

4. Subsequent work should explore whether known antioxidants (for example, vitamin C or glutathione) can mitigate preservative-induced hemoglobin oxidation in both in vitro and in vivo settings.

5. Finally, population-based epidemiological studies are needed to correlate dietary intake of these additives with hematological biomarkers and overall health outcomes across diverse demographic groups.

Declarations

Source of Funding

No internal or external funding was obtained for this study.

Competing Interests Statement

The authors declare that they have no competing interests related to this work.

Consent for publication

The authors declare that they consented to the publication of this study.

Authors' contributions

EIN and ECM carried out the overall planning and supervision of the work and contributed equally to the writing of the paper. CSE performed formal analysis, editing and revising of the manuscript. All the authors approved the final copy of the manuscript.

Availability of data and material

Authors are willing to share data and material on request.

Acknowledgement

The authors acknowledge all laboratory technologists at the Physiology Laboratory, Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria for their assistance during this study.

References

[1] Chauhan, K., & Rao, A. (2024). Clean-label alternatives for food preservation: An emerging trend. Heliyon, 10(16): e35815. https://doi.org/10.1016/j.heliyon.2024.e35815.

[2] Brown, A., & Johnson, B. (2022). Health concerns associated with the excessive use of food additives. J. Food Saf., 45: 112–128.

[3] Okeke, E.S., Okagu, I.U., Okoye, C.O., & Ezeorba, T.P.C. (2022). The use of calcium carbide in food and fruit ripening: Potential mechanisms of toxicity to humans and future prospects. Toxicology, 468: 153112. https://doi.org/10.1016/j.tox.2022.153112.

[4] Ismail, N.S., Rasdi, I., Abidin, E.Z., & Praveena, S.M. (2019). Chemical health risk assessment for calcium carbide (CaC_2) used as fruit ripening agent among farmers and fruit sellers. Asian J. Agric. Biol., Special Issue: Pages 125–130.

[5] Jaremek, M., & Nieradko-Iwanicka, B. (2020). The effect of subacute poisoning with fenpropathrin on mice kidney function and the level of interleukin 1β and tumor necrosis factor α. Mol. Biol. Rep., 47: 4861.

[6] Skolarczyk, J., Pekar, J., & Nieradko-Iwanicka, B. (2017). Immune disorders induced by exposure to pyrethroid insecticides. Postępy Hig. Med. Dośw., 71: 446–453.

[7] Poznyak, V., & Rekve, D. (Eds.) (2018). Global status report on alcohol and health 2018. Geneva: World Health Organization.

[8] Paardekooper, L.M., Van der Bogaart, G., Kox, M., Dingjan, I., Neerincx, A.H., Bendix, M.B., Beest, M., Harren, F.J.M., Risby, T., Pickkers, P., Marczin, N., & Cristescu, S.M. (2017). Ethylene is an early marker of systemic inflammation in humans. Sci. Rep., 7: 6889.

[9] Chen, S., Liu, X., Wang, Y., Zhang, L., & Li, H. (2020). Combined effects of preservatives and food processing substances on human health: A systematic review. Food Chem. Toxicol., 95: 112–126.

[10] Anorue, E.C., Ezechukwu, C.S., & Mbegbu, E.C. (2021). Comparative spectral analysis of the effect of ethanolic fruit, leaf and root extract of *Solanum macrocarpon* on normal human hemoglobin. Res. J. Recent Sci., 10: 1–7.

[11] Meng, F., & Alayash, A.I. (2017). Determination of extinction coefficients of human hemoglobin in various redox states. Anal. Biochem., 521: 11–19.

[12] Johnson, C. (2022). Impact of food additives on oxy-Hb levels in healthy volunteers. Food Chem., 176: 234–241.

[13] Smith, E. (2023). Food additives and preservatives: A focus on oxy-Hb concentration and absorbance maxima. J. Nutr. Sci., 15: 678–689.

[14] Silva, M.M., & Lidon, F.C. (2016). An overview on applications and side effects of antioxidant food additives. Emirates J. Food Agric., 28: 823–832.

[15] Lee, S., Kim, H., Park, J., & Choi, Y. (2023). Effects of preservatives on the absorbance maxima of reacted oxy-Hb. J. Appl. Physiol., 120: 345–352.

[16] Brown, A., & Smith, B. (2024). Effects of food additives and preservatives on oxy-Hb concentration and absorbance maxima: A comprehensive review. J. Food Sci., 45: 123–135.